Genetic Heterogeneity in Lamellar Ichthyosis

To the Editor:
It is reasonable to expect that lamellar ichthyosis is genetically heterogeneous. We believe, however, that the analysis presented in the recent report by Huber *et al* (1995) in the November issue of the *Journal of Investigative Dermatology* does not provide conclusive evidence in support of this hypothesis.

We disagree with the definition of the sequence differences in the gene for transglutaminase-1 as "polymorphisms." Polymorphisms are changes in a DNA sequence that are seen in more than 1% of the population. It is usual to check 100 normal alleles to determine the frequency of a given DNA change giving the investigator about a 95% chance of finding a rare polymorphism in the population if it exists. Huber *et al* checked 20 alleles, one-fifth the appropriate number, reducing the power to detect a polymorphism by five times. Furthermore, they did not observe either of their DNA sequence changes in these 20 alleles. Therefore, there is no evidence to support calling these changes "polymorphisms."

Huber *et al* reported two sequence variations in TGM1 in their LI patients. The nucleotide positions given do not correspond to published TGM1 sequences. Moreover, in one of these variations, the nucleotide upstream from the splice acceptor site of intron 5, described in patient LI-4.5 as changed from G to C, is, in fact, a C in the published Genbank sequences (M98447, M86360, M83227). To add to the confusion, "In this issue. . . " states that "the DNA shows no deviation from published sequence."

If there actually was a change in a nucleotide upstream from the splice acceptor site of intron 5, a small change in the primary structure of transglutaminase-1 mRNA and protein could result which likely would not have been detected by the Northern and Western analyses shown (e.g., skipping of exon 6, deleting 108 nucleotides and 33 in-frame codons).

The table showing transglutaminase activity in keratinocytes is missing information necessary to interpret the data. Can the assay used detect a profound decrease in activity? Can the assay identify a heterozygote? What is the evidence that a cytosolic:membrane ratio of 0.11 is not significantly different from 0.05 (a difference of 2X)? Or that cytosolic activity of 29.3 $\pm$ 2.4 is not significantly different from 11.1 $\pm$ 6.5, when the 95% confidence interval around each of these estimates do not overlap? Although the authors perform no statistical tests, these results are highly suggestive of a difference between the normal and patient samples.

The term "sporadic" to describe LI in these families is used incorrectly. Sporadic means either that the affected individuals arose from a new mutation (e.g., a child with epidermolytic hyperkeratosis whose parents are both unaffected) or that the individual represents a nongenetic case in a family where other cases have a genetic etiology (e.g., in a family with hereditary breast cancer, a woman who does not carry the breast cancer gene but develops breast cancer for other reasons is a sporadic case). The correct term for a family in which only a single person is affected by an inherited disease is "simplex." Simplex families are not uncommon among lamellar ichthyosis families. The cases in simplex families are not sporadic.

Finally, what is the basis for the diagnosis of lamellar ichthyosis in the patients? Huber *et al* state that "both presented as generalized erythematous LI." Two types of autosomal recessive ichthyosis, lamellar ichthyosis and congenital ichthyosiform erythroderma, can be distinguished clinically. The paucity of clinical information given about these two patients precludes a precise diagnosis. If the issue of genetic and clinical heterogeneity in the ichthyoses is to be resolved, appropriate clinical descriptions must be presented along with molecular and cell biologic results.

Sherri J. Bale
Chief, Genetic Studies Section, LSB

John G. Compton
Staff Scientist, Genetic Studies Section, LSB

Laura J. Russell
Sr. Staff Fellow, Genetic Studies Section, LSB

John J. DiGiovanna
Head, Dermatology Clinical Research Unit

Intramural Research Program
National Institute of Arthritis and Musculoskeletal and Skin Disease
National Institutes of Health
6 Center Dr., MSC-2757
Bethesda, MD 20892-2757

REFERENCE

Huber M, Rettler I, Bernasconi K, Wyss M, Hohl D: Lamellar ichthyosis is genetically heterogeneous—cases with normal keratinocyte transglutaminase. *J Invest Dermatol* 105:653–654, 1995

Reply:
We thank Drs Bale, Compton, Russell, and DiGiovanna for their interest in our work on autosomal recessive lamellar ichthyosis (LI) [1].

For the nucleotide sequence numbering we refer correctly to the paper of Phillips *et al* describing the genomic structure of keratinocyte transglutaminase, where the putative transcription start site has been numbered as +1 (see Fig 2 [2]).

We agree that the heterozygous sequence change in patient LI-4.5 at position +3365 should read in fact as a C to G change. We apologize for this typing error. We also have no problem with the statement that a substitution at position −3 of the splice acceptor site of intron 5 could potentially be harmful. It is well known that the C nucleotide at this position of splice acceptor sites is highly conserved [3]. Such a potential deletion of exon 6 would hardly be detected by northern analysis. We disagree, however, that the respective protein changes would not have been identified by western techniques. In support of this notion, the same group at the National Institutes of Health published an outstanding study on the structure of transglutaminase K (TGK) demonstrating clear mobility differences on western blots among TGK mutants differing in only 53 or 45 amino acids at the amino-terminal end (see *lanes 1, 3,* and *5* in [4]). Another excellent paper on polymorphic K1 alleles from the same National Institutes of Health group even describes detectable mobility differences of K1 proteins varying in as few as 7 deleted amino acids (see Fig 4 in [5]). Thus, there is no doubt that our careful western analysis (see Fig 2 in [1]) would have easily

0022-202X/96/$10.50

detected a 4- to 5-fold larger deletion of 33 amino acids, that is, a mobility shift of approximately 3.3 kDa. Furthermore, we observed a normal TG enzyme activity in LI-4.5 (see Table I in [1]). A potential heterozygous carrier would be expected to show a decrease of about 50% from the normal values. In short, there is clear and conclusive evidence to reject the hypothesis of exon skipping or other mRNA changes affecting TGK structure/function in LI-4.5 brought forward by Bale, Compton, Russell, and DiGiovanna.

We have shown earlier that our assay based on a published protocol [6] permits detection of profound decreases in TG activity [7]. As is stated in Table I of our paper [1], we have measured the TG activity in duplicates from two different cell passages. If we calculate the cytosolic:membrane ratio from the two cell passages we see no significant difference between the patients and normal controls (LI-4.5, 0.07 ± 0.04; LI-5.3, 0.05 ± 0.04; N1, 0.11 ± 0.02; N2, 0.09 ± 0.02). Thus, we do not understand why the authors compare the value of 11.1 ± 6.5 with only the one of N1 and not also with the one of N2, where the 95% confidence interval does overlap? Further data from six normal controls measured in our lab show that the cytosolic TG activity ranges from 100 to 500 pmol of putrescine incorporated per mg hour. LI-4.5 and LI-5.3, with 241.1 and 207.5 pmol/mh hour, respectively, are well within this range; and the same is true for the membrane activities. Therefore, we strongly disagree with the authors and see no evidence of biochemical difference between our patients and normal controls.

Further evidence that LI is genetically heterogeneous has now been provided by two other groups showing that only in 10 out of 23 families [8] and in 10 out of 27 families [Hennies H, Mackova A, Ehrig T, Küster W, Reis A: Autosomal recessive lamellar ichthyosis: detection of mutations in the TGM1 gene and molecular heterogeneity. *Jahrestagung der ADF*, Abstract V26 1996, 9] respectively, is LI tightly linked to the TGM1 locus. Both groups could not identify clear-cut clinical pictures corresponding to a particular genetic linkage. This raises the question whether the concept of only two distinct forms of autosomal recessive ichthyoses, lamellar ichthyosis and congenital ichthyosiform erythroderma, is still valuable and will survive. At the least, there is no doubt that neither erythroderma nor the presentation as collodion baby provide by themselves a reliable tool for pathogenic classification [7, 8, Hennies *et al. Jahrestagung der ADF*, Abstract V26 1996, 9]. In our own series of over 25 LI families, we recognize a tendency toward larger scales in TGK-deficient LI patients, however, this phenotype does not allow pathogenic classification on clinical grounds (D. Hohl, unpublished observations). Thus, we fully share the opinion of Howard Baden that the assignment of patients into categories must await the elucidation of the fundamental pathophysiology [10]. Once all the different causes of LI will be identified, their specific clinical pictures may or may not become more apparent.

Whether patients are called sporadic or simplex cases and sequence differences polymorphisms or rare variants does not add any valuable information regarding the question whether LI is pathogenetically heterogeneous or not. Such semantic differences of common language used either by molecular geneticists (see M. Thompson *et al*: "Genetics in Medicine") or by molecular biologists (see B. Lewin: "Genes") rather reflect the profoundly different approaches that our two groups have taken in seeking the molecular origin of epidermal genodermatoses. Despite the undisputed power of gene mapping [11], decent biochemical and molecular work still has its place, as has been demonstrated [7]. In fact, we are convinced that future routine diagnostic tools to identify TGK-deficient LI will be based on biochemical assays rather than on genetic linkage.

Daniel Hohl
Marcel Huber
Laboratory of Skin Biology
Department of Dermatology-CHUV
CH-1011 Lausanne, Switzerland

REFERENCES

1. Huber M, Rettler I, Bernasconi K, Wyss M, Hohl D: Lamellar ichthyosis is genetically heterogeneous—cases with normal keratinocyte transglutaminase. *J Invest Dermatol* 105:653–654, 1995
2. Phillips MA, Stewart BE, Rice RH: Genomic structure of keratinocyte transglutaminase. *J Biol Chem* 267:2282–2286, 1992
3. Krawzak M, Reiss J, Cooper D: The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. *Hum Genet* 90:41–54, 1992
4. Kim SY, Kim IG, Chung SI, Steinert PM: The structure of the transglutaminase 1 enzyme—deletion cloning reveals domains that regulate its specific activity and substrate specificity. *J Biol Chem* 269:27979–27986, 1994
5. Korge BP, Compton JG, Steinert PM, Mischke D: The two size alleles of human keratin-1 are due to a deletion in the glycine-rich carboxyl-terminal-V2 subdomain. *J Invest Dermatol* 99:697–702, 1992
6. Lichti U, Ben T, Yuspa SH: Retinoic acid-induced transglutaminase in mouse epidermal cells is distinct from epidermal transglutaminase. *J Biol Chem* 260:1422–1426, 1985
7. Huber M, Rettler I, Bernasconi K, Frenk E, Lavrijsen S, Ponec M, Bon A, Lautenschlager S, Schorderet D, Hohl D: Mutations of keratinocyte transglutaminase in lamellar ichthyosis. *Science* 267:525–528, 1995
8. Parmentier L, Blanchet-Bardon C, Nguyen S, Prud'homme J-F, Dubertret L, Weissenbach J: Autosomal recessive lamellar ichthyosis: identification of a new mutation in transglutaminase 1 and evidence for genetic heterogeneity. *Human Mol Gen* 4:1391–1395, 1995
9. Reis A, Hennies H, Mackova A, Ehrig T, Küster W: Molecular heterogeneity and mutation detection in autosomal recessive lamellar ichthyosis. *Am J Hum Genet* 57:A46, 1995
10. Bernhardt M, Baden H: Report of a family with an unusual expression of recessive ichthyosis. *Arch Dermatol* 122:428–433, 1986
11. Russell LJ, DiGiovanna JJ, Rogers GR, Steinert PM, Hashem N, Compton JG, Bale SK: Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. *Nature Genet* 9:279–283, 1995

HIV-1 Transmission to Lymphoid Cells from Epidermal Cell Cultures Derived from Skin Biopsies of AIDS Patients

To the Editor:

Normal human keratinocytes can be isolated from skin biopsies and, cultivated under proper conditions, can reconstitute cohesive sheets of epithelium closely resembling the normal human epidermis *in vitro* [1]. Epithelial sheets at the second passage of culture are widely used for auto- and allografting on patients. Allografts, which can be stored frozen, are successfully used for the treatment of deep partial skin burns and limited skin defects [2]. Allografts do not take permanently but stimulate re-epithelization by resident keratinocytes through secretion of polypeptides in the wound fluids [3]. It has been demonstrated that epidermal Langerhans cells as well as cultured keratinocytes are infectable by human immunodeficiency virus-1 (HIV-1) [4,5]. It has been also reported that the virus can be retained in a semilatent form by keratinocyte monolayers and rescued to full replication by a proper target even after multiple passages in culture (5). These observations prompted us to inves-

Manuscript accepted for publication March 19, 1996.